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Physiological and growth responses to pruning season and intensity of hybrid poplar



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ABSTRACT

Pruning is one of the silvicultural practices recommended for poplar plantations in order to produce clearwood. Common wisdom proposes pruning during the summer season, however little is known on trees' physiological responses to pruning season and intensity. This study aimed at determining the effects of pruning season and intensity on growth and physiology of four hybrid poplar clones. Using 3 years old trees, we compared nine combinations of pruning season (fall, spring and summer) and intensity (unpruned, 1/3 and 2/3 of crown length pruned). We found that pruning increased net photosynthesis of residual foliage, nitrogen foliar concentrations and reduced total non-structural carbohydrates reserves of roots. Leaf carbon isotopic ratios ($\delta^{13}\text{C}$) of pruned trees decreased compared to unpruned trees, reflecting their greater stomatal conductance and suggesting that pruned trees could have greater drought stress resistance. Two growing seasons after pruning, 1/3 pruned trees had reduced height growth except for those pruned in summer. Regardless of pruning season, pruning 2/3 of the live crown reduced both breast height diameter and height 2 years after treatments. Summer pruning increased foliar nitrogen concentrations, stomatal conductance and decreased leaf carbon isotopic ratios ($\delta^{13}\text{C}$) more than for trees pruned in fall or spring, suggesting better capacities to recover from pruning. We concluded that removal of 1/3 of the lower crown in summer was the best option to produce clear wood without compromising growth rates and physiological processes in hybrid poplar.

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1. Introduction

The area of primary forests has decreased by more than 40 million ha between the years of 2000 and 2010 (FAO, 2010). Establishment of fast growing plantations is considered as the main tool to produce more wood on reduced land areas by preserving the remainder of native forests while ensuring long-term timber supplies (Heilman, 1999; Anderson and Luckert, 2007). To maintain high growth rates and/or increase products value, fast growing plantations require silvicultural interventions right from stand establishment to the final cut. The timing and intensity of silvicultural operations can significantly affect profitability, and also the physiology and growth of trees. Pruning treatments to remove branches from the lower crown are usually done to improve the shape of trees during the establishment period and/or to create knot free wood and increase the value of boles (Hubert and Courraud, 1994).

Most pruning prescriptions are based on empirical data combining operational needs with tree growth responses (Keller, 1979; Uotila and Mustonen, 1994; Neilsen and Pinkard, 2003; Shock et al., 2003). As a consequence, pruning prescriptions tend

to be conservative and designed to ensure that growth is not affected by a particular level of pruning under most conditions (Pinkard and Beadle, 2000). Pruning intensity (or height to which branches are removed) is usually determined by the desired length of clearwood logs for saw and veneer (Montagu et al., 2003). Recommendations for hybrid poplars in Canada suggest reaching a 6–7 m clear bole, in 3–5 lifts depending on tree growth, only removing one-third of the live crown at each lift (Boysen and Strobl, 1991; van Oosten, 2006; Fortier et al., 2011).

Trees develop physiological responses to compensate pruning or defoliation such as compensatory photosynthesis, which is defined as an increase in the photosynthetic rates of foliage of partially defoliated plants relative to foliage of the same age on undefoliated plants (Nowak and Caldwell, 1984; Hart et al., 2000). This mechanism also triggers an increase in the rate of leaf development and in the longevity of existing leaves (Pinkard and Beadle, 1998a). An increased utilization of carbohydrate reserves is also a compensatory mechanism to support the production of new foliage (Tschaplinski and Blake, 1994), such as observed in the needles of pruned *Pinus sylvestris* (Långström et al., 1990). It has also been observed that pruning increased nitrogen concentrations in the remaining leaves of pruned trees (Pinkard et al., 1998; Turnbull et al., 2007). Such improved nutrient status in residual tissue may result directly from modified root-shoot ratios (McNaughton,

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1983). Moreover, pruning appeared to increase leaf water potential by reducing leaf area and therefore transpiration (Elfadl and Luukkanen, 2003). These responses depend on species (Reich et al., 1993) and generally increase with pruning intensity (Medhurst et al., 2006; Alcorn et al., 2008).

Physiological processes are subjected to seasonal variations (Kozłowski and Pallardy, 1997), and physiological responses to pruning could also vary according to the pruning season. However, the literature is devoid of recommendations based on physiological responses to pruning season. Most recommendations pertaining to pruning season are based on wound healing, insects or fungi damages at wound sites and the extension of wood discoloration (Shigo et al., 1978; Shigo, 1985; Lonsdale, 1993; Soutrenon, 1995; Dujesiefken and Stobbe, 2002; Pinkard et al., 2004; Dujesiefken et al., 2005). Pruning during the dormant season is often discouraged because wound closure is slower, tree defenses are less operational and wounds are less effectively compartmentalized (Lonsdale, 1993; Dujesiefken et al., 2005). Hence, it has been suggested that the best time to prune was at the end of winter when the tree defence system is about to become active, but before carbohydrate reserves are depleted by leaf flushing (Shigo, 1989), resulting in quick wound healing without spread of potential diseases (Soutrenon, 1995). Then again, prescriptions for pruning season depend on species and climate. In Europe, literature suggests pruning poplars at the end of the summer (Bonduelle, 1971; Hubert and Courraud, 1994), while in Canada where the growing season is shorter, it is mostly recommended to prune poplars in late spring or early summer (Boysen and Strobl, 1991; van Oosten, 2006; Fortier et al., 2011). These recommendations, however, do not seem to be based on any physiological rationale or experimental data, but rather on conventional wisdom and traditional practice.

The main objective of this study was to assess physiological and growth responses to pruning season and intensity in different hybrid poplar clones. Gas exchange, root total non-structural carbohydrates and foliar nitrogen concentrations, and water stress were evaluated after three seasonal pruning (fall, spring or summer) and three pruning intensities (unpruned, 1/3 or 2/3 of crown length pruned). We anticipated that removal of foliage and branches would trigger gas exchange responses, modifications of carbohydrate and nitrogen (N) stocks, and a reduction in transpiration possibly enhancing trees' resistance to drought stress. Such responses are also likely to be affected by the season when trees are pruned, in turn affecting tree growth and vigour. Since summer-pruned trees keep the totality of their photosynthetic area during the first part of the growing season, we expected greater root carbohydrate reserves hence stronger physiological responses to compensate for the loss of foliage. We also predicted that the magnitude of the response would increase with pruning intensity. A better understanding of physiological responses to pruning could help us recommend more appropriate silvicultural practices.

2. Materials and methods

2.1. Study site

The study was conducted in a plantation established in 2007 at the New Liskeard Agricultural Research Station of Guelph University in North-Eastern Ontario (47°52'N, 79°66'W). The area is part of Haileybury Clay forest section of the Great Lakes-St. Lawrence Forest Region (Rowe, 1972). Annual average precipitation for the region between years 1971 and 2000 averaged 819 mm (rainfall 625 mm, snowfall 195 cm) with an average daily temperature of 2.8 °C and an average of 2605 degree-days above 0 °C (Ville-Marie station 47°21'N, 79°26'W) (Environment Canada, 2011). The regional

surficial geology is characterized by lacustrine clays and sands from post-glacial Lake Barlow (Rowe, 1972), and soil type was a Humic Gleysol (Canada Soil Survey Committee, 1987).

The site was ploughed in October 2006 and cross-cultivated with disks followed by herbicide application in spring 2007 before planting at a 3.5 m × 3.5 m spacing (816 stems ha⁻¹). Four hybrid poplar clones were chosen based on their different parentage: 1079 (*Populus* × *jackii* (*P. balsamifera* × *P. deltoides*)), 747210 (*P. balsamifera* × *P. trichocarpa*), 915319 (*P. maximowiczii* × *P. balsamifera*) and DN2 (*P. deltoides* × *P. nigra*). The planting stock consisted of standardized dormant 1 year old bareroot trees. Plantation was followed by local fertilization with 18-23-18 (N-P-K, 110 g tree⁻¹). Yearly weed control was done by cross cultivation with disks followed by herbicide application (Roundup™).

Trees were planted in a randomized block design with three blocks (replicates), each containing four plots of 100 trees (10 rows × 10 trees), one for each clone. Each plot was randomly divided into three pruning seasons (October 2009 = fall, March 2010 = spring or June 2010 = summer) and three intensities (unpruned, 1/3 or 2/3 of crown length removed). One row of trees was used for each treatment combination (9). Five trees of each row were randomly selected for the study (pseudo-replicates; $n = 540$). If epicormic branches sprouted from the stem of pruned trees during the two growing seasons after pruning, they were removed with respect to the pruning treatment they were assigned.

2.2. Field sampling

Diameter at breast height (*dbh*) and total height (*H*) were measured in fall 2009 and at the end of the 2010 and 2011 growing seasons. Stem volume was calculated from the following equation developed by Boysen and Strobl (1991) and then converted into volume per hectare:

$$V = (\exp(-1.064079 + 1.562891 * \ln(D) + 0.101423 * \ln(H)) * 1.013914) / 1000$$

where *V* is the stem volume in m³, *D* the *dbh* in cm and *H* the tree height in m.

Instantaneous net photosynthesis and stomatal conductance were measured at weekly intervals with a CIRAS-2 portable infra-red gas analyzer (PP Systems, Amesbury, MA). Repeated measurements on the same tree were done weekly between the second and fifth week following the summer pruning. Measures were done on the youngest fully expanded leaves located in the upper third of the crown. Trees were selected randomly and one measure was made for each treatment i.e., $n = 108$ for each week, except at week 2 where only two blocks (replicates) were measured due to a battery problem. One root sample per treatment combination ($n = 108$) was collected for determination of total non-structural carbohydrates (TNC) concentrations at the end of august 2010 by cutting a disk from one of the main roots. Root disks of similar size were collected 30 cm from the trunk from roots directly connected to the base of the tree.

Four to six leaves were collected from the upper third of the canopy of each tree in July (5 weeks after the summer pruning) for nitrogen ($n = 108$) and carbon isotopic discrimination ($\delta^{13}\text{C}$) analyses. For trees pruned in summer, two types of leaves were distinguished for the $\delta^{13}\text{C}$ analysis: leaves formed before or after pruning ($n = 132$).

2.3. Laboratory work

Roots samples for TNC analysis were oven-dried at 65 °C until constant weight, and ground to pass a 40 mesh screen of a Wiley mill (model 3383-L10; Thomas Scientific, Swedesboro, NJ). Soluble

sugars were extracted by boiling three times in 80% ethanol at 95 °C. Phenol–sulfuric acid was used to analyze the ethanol extract for total sugar concentrations. Starch was digested using an enzyme mixture of α -amylase and amyloglucosidase followed by the colorimetric measurement of the glucose hydrolysate using a peroxidase–glucose oxidase–*o*-dianisidine reagent (Chow and Landhausser, 2004).

Leaf samples were oven-dried at 65 °C until constant weight then ground finely with a Mixer Mill MM301 ball grinder (Retsch Inc., Newtown, PA). Carbon isotopic composition ($\delta^{13}\text{C}$) and total nitrogen concentration were done at the Soil Biochemistry Laboratory, University of Alberta, Edmonton, Canada. Samples were flash combusted at 1800 °C using a Costech ECS 4010 Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA) coupled to a Finnigan Conflo III and a Finnigan Delta Plus Advantage Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Electron Corporation, Mississauga, Ont.). The resulting gas were separated on a 2 m \times 6 mm OD stainless steel Porapak Q5 80/100 mesh packed chromatographic column and detected quantitatively by a Thermal Conductivity Detector.

The isotopic composition of the samples was calculated from:

$$\delta^{13}\text{C} = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$$

where R_{sample} and R_{standard} are the ratios of $^{13}\text{C}/^{12}\text{C}$ in the sample and standard, respectively. Spring wheat grain was used as the working standard, with a carbon isotope composition of -23.47‰ relative to Pee Dee Belemnite.

3. Statistical analyses

Statistical analyses were conducted with “R” software version 2.15.1 (R Development Core Team, 2012). Data were analyzed with linear mixed-effects models using the “lme” function from the “nlme” package (Pinheiro et al., 2012). We used Backward model selection to determine the most suitable model for each response variable; height (H model), *dbh* (D model), net photosynthesis (PN model), stomatal conductance (GS model), total non-structural carbohydrates (TNC model), leaf nitrogen (N model) and leaf carbon isotopic ratio (Delta_1 model). Backward selection tested the model containing all the variables (global model) and removed the least significant variable until all variables included in the model were significant at $p < 0.05$. Then, we used multiple comparisons of means (Tukey’s tests) to reveal differences among treatments with the “multcomp” package (Hothorn et al., 2008). A significance level of $p < 0.05$ was used. Unlike traditional analysis of variance, linear mixed models incorporate both fixed and random effects. Hence, to reckon on the experiment design, all models included random effects that consisted of block, and clone nested in block. Parameter tree was incorporated as a random effect in the model sets with repeated measures (models D, H, GS and PN). Growth data were analyzed including initial height and diameter at breast height as covariates. To take into account the influence of root diameter on total non-structural carbohydrate

concentrations (DesRochers et al., 2002), root diameter was included in the TNC model as a covariate. Stomatal conductance was log-transformed to normalize residuals and to improve homoscedasticity. However, results and predicted values are presented in back-transformed units. For $\delta^{13}\text{C}$ analyses of leaves of summer-pruned trees (intensities 1/3 and 2/3 only), a separate data set (DELTA_2 model) was created to account for the two types of leaves: leaves formed before (S_1) or after (S_2) pruning (old vs new leaves).

4. Results

4.1. Growth responses

Mean tree height and *dbh* of the plantation after five growing seasons was 7.64 m and 9.1 cm, respectively (Table 1). Trees of clone DN2 were the largest with a mean height of 9.09 m and a mean *dbh* of 11.54 cm. Conversely, trees of clone 747210 were the smallest with a mean height of 6.07 m and a mean *dbh* of 6.5 cm (Table 1). There were no significant interactions between clone and the pruning treatments (Table 2) indicating that clones responded similarly to treatments.

A significant interaction between pruning intensity and season (Table 2) indicated that height growth decreased with pruning intensity, except when trees were pruned at intensity 1/3 in summer (Fig. 1a). There was also an interaction between year and pruning intensity (Table 2), showing that height decreased in 2010 only for trees pruned at intensity 2/3 (Fig. 2a) while, 2 years after pruning (in 2011), a reduction in tree height was also observed for trees pruned at intensity 1/3 (Fig. 2a).

There was also a significant interaction between pruning intensity and season for *dbh* (Table 2), showing that pruning had no effect on *dbh* growth for trees pruned in fall or spring, while pruning at intensity 2/3 decreased *dbh* of trees pruned during summer compared to unpruned trees (Fig. 1b). Differences in *dbh* observed at the end of the first growing season (2010) persisted for the next growing season (2011), except for trees pruned at intensity 2/3 for which a greater decrease in *dbh* was observed after the second growing season (Fig. 2b).

4.2. Net photosynthesis

Instantaneous net photosynthesis (Pn) measurements were greater for trees pruned at intensities 1/3 and 2/3 compared to unpruned trees (Fig. 3a), while pruning season did not affect net photosynthesis (Table 3). Net photosynthesis varied between clones (Table 3); Clones 747210 ($13.61 \mu\text{mol m}^{-2} \text{s}^{-1}$) and 915319 ($13.49 \mu\text{mol m}^{-2} \text{s}^{-1}$) had greater photosynthetic rates than clone DN2 ($10.09 \mu\text{mol m}^{-2} \text{s}^{-1}$), while clone 1079 was intermediate ($11.17 \mu\text{mol m}^{-2} \text{s}^{-1}$). Net photosynthesis values were similar for the first 4 weeks after summer pruning, while they strongly increased over all treatments at week five ($+5.419 \mu\text{mol m}^{-2} \text{s}^{-1}$; Table 3).

Table 1

Mean tree height, diameter at breast height (*dbh*), and estimated volume per hectare for each clone. Standard errors of the mean are indicated in parentheses. Values labeled with the same letter within a year are not significantly different.

Clone	Height (m)			<i>dbh</i> (cm)			Volume ($\text{m}^3 \text{ha}^{-1}$)		
	2009	2010	2011	2009	2010	2011	2009	2010	2011
1079	4.47 (0.67) ab	5.82 (0.66) b	7.46 (0.67) b	4.28 (1.03) b	6.41 (1.17) b	8.55 (1.18) b	3.3 (1.43)	6.3 (2.09)	10.3 (2.57)
747210	3.78 (0.74) a	4.72 (0.81) a	6.07 (0.97) a	3.07 (0.91) a	4.81 (1.16) a	6.5 (1.38) a	2.0 (1.07)	4.0 (1.83)	6.5 (2.69)
915319	5.20 (0.91) b	6.33 (0.87) bc	7.92 (0.89) b	5.47 (1.68) c	7.51 (1.74) bc	9.71 (1.89) b	5.0 (2.85)	8.3 (3.60)	12.5 (4.66)
DN2	5.07 (0.75) b	7.00 (0.84) c	9.09 (0.83) c	5.41 (1.49) bc	8.33 (1.67) c	11.54 (2.07) c	4.9 (2.43)	9.7 (3.56)	16.6 (5.49)

Table 2Best fit models chosen with backward selection for *dbh* (D) and height (H) analysis of variance. Statistically significant values ($p < 0.05$) are given in bold.

Variable	D		H	
	Estimate (SE)	p-Value	Estimate (SE)	p-Value
(Intercept)	1.949 (0.186)	<0.001	1.765 (0.134)	<0.001
2009	1.053 (0.023)	<0.001	0.921 (0.020)	<0.001
Year (2011)	2.391 (0.161)	<0.001	1.717 (0.024)	<0.001
Clone (747210)	−0.609 (0.203)	0.024	−0.595 (0.121)	0.003
Clone (915319)	−0.162 (0.203)	0.455	−0.173 (0.121)	0.203
Clone (DN2)	1.224 (0.204)	0.001	0.845 (0.121)	<0.001
Intensity (1)	0.234 (0.122)	0.055	−0.162 (0.058)	0.005
Intensity (2)	−0.175 (0.121)	0.150	−0.270 (0.058)	<0.001
Season (spring)	−0.095 (0.092)	0.302	−0.028 (0.044)	0.529
Season (summer)	−0.048 (0.091)	0.598	−0.004 (0.043)	0.934
Intensity (1):season (spring)	−0.170 (0.163)	0.298	−0.044 (0.078)	0.575
Intensity (2):season (spring)	0.163 (0.162)	0.314	0.179 (0.078)	0.021
Intensity (1):season (summer)	−0.384 (0.156)	0.014	0.203 (0.075)	0.007
Intensity (2):season (summer)	−0.182 (0.156)	0.244	0.203 (0.075)	0.761
Year (2011):intensity (1)	−0.043 (0.090)	0.636	−0.071 (0.042)	0.086
Year (2011):intensity (2)	−0.257 (0.090)	0.004	−0.155 (0.041)	<0.001

Notes: SE is standard error of the mean. 2009 is the initial growth measurements (*dbh* for model D, and height for model H). Year is the year of growth measurement, Intensity is the pruning intensity (1 = 1/3, 2 = 2/3 crown length), Intensity:season is the interaction between pruning intensity and pruning season and Year: Intensity is interaction between year of measurement and pruning intensity. Letters or numbers indicated in brackets indicate the type considered by the model. Combined with the values (column estimate) it allows to calculate the predicted values; for example in model D, *dbh* at Year (2011) for Clone (747210) pruned at Intensity (1) in spring was $1.949 + 1.053 * 4.532$ (mean diameter) + $2.391 - 0.609 + 0.234 - 0.095 - 0.170 - 0.043$.

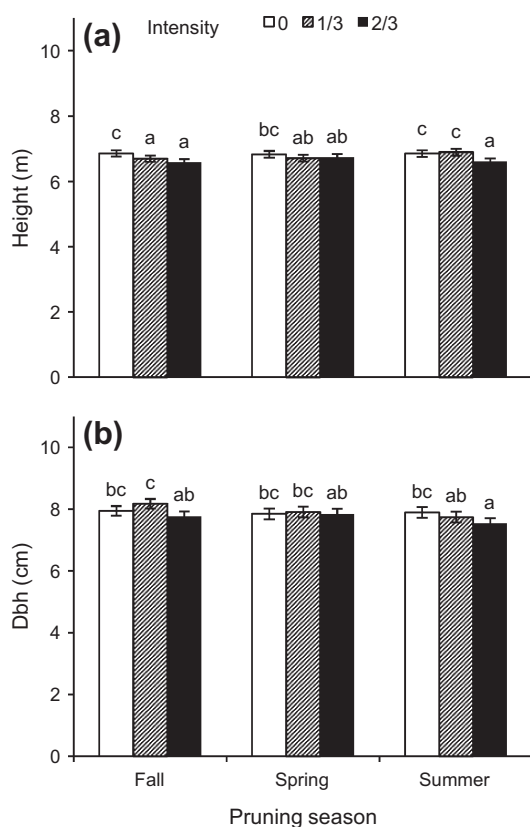


Fig. 1. Predictions for height (a) and *dbh* (b) across pruning intensities and seasons. Errors bars are standard errors of the mean. Bars labeled with the same letter within a graph are not significantly different (Tukey's test at $p < 0.05$). Note that values were computed for clone DN2 in 2010.

4.3. Total non-structural carbohydrates (TNC)

Root TNC concentrations were lower at pruning intensities 1/3 and 2/3 compared to unpruned trees (Fig. 3b). Parameters clone and pruning season did not appear in the selected model during the statistical analyses of the data, hence had no effect on root TNC concentrations (Table 3).

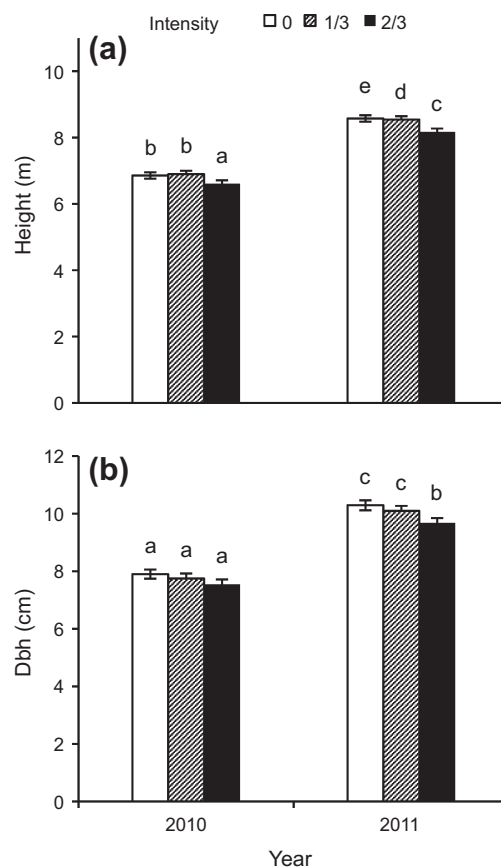


Fig. 2. Predictions for height (a) and *dbh* (b) across pruning intensities, one and two growing seasons following pruning. Errors bars are standard errors of the mean. Bars labeled with the same letter within a graph are not significantly different (Tukey's test at $p < 0.05$). Note that values were computed for clone DN2 pruned in summer.

4.4. Carbon isotopic discrimination

As pruning intensity increased, $\delta^{13}\text{C}$ values decreased (Fig. 3c). For trees pruned in summer, there was a significant interaction

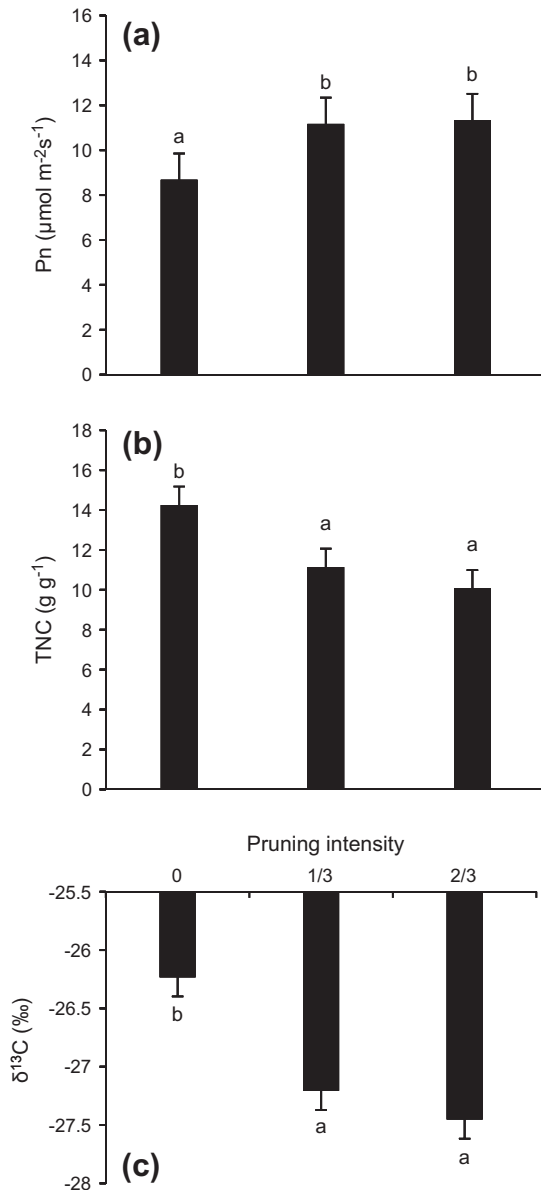


Fig. 3. Predictions for instantaneous net photosynthesis (Pn); (a), total non-structural carbohydrate (TNC) concentration (b); and leaf carbon isotopic discrimination ($\delta^{13}\text{C}$); (c) across pruning intensities. Errors bars are standard errors of the mean (SE). Bars labeled with the same letter within a graph are not significantly different (Tukey's test at $p < 0.05$). Note that values were computed for clone 1079 (a), at week 2 (a) (second week of gas exchange measurements) and for mean root diameter (b).

between pruning intensity and the age of leaves (Table 3), showing that $\delta^{13}\text{C}$ values of old leaves (S₁) were similar for each pruning intensity, while values of newly formed leaves (S₂) were significantly lower at intensity 2/3 compared to 1/3 (Fig. 4). Values of $\delta^{13}\text{C}$ also differed within each pruning intensity for old (S₁) and newly formed (S₂) leaves; mean $\delta^{13}\text{C}$ increased from old to newly formed leaves in the 1/3 pruning intensity, while it decreased for the 2/3 pruning intensity (Fig. 4).

4.5. Stomatal conductance

There was a strong increase in stomatal conductance (G_s) 5 weeks after summer pruning as it increased by 79%, 108% and 114% compared to weeks 2, 3 and 4 respectively (Table 4). Stomatal

Table 3

Best fit models chosen with backward selection for instantaneous net photosynthesis (PN), total non-structural carbohydrate concentration in roots (TNC) and leaf carbon isotopic discrimination (DELTA_1 and DELTA_2) analysis of variance. Statistically significant values ($p < 0.05$) are given in bold.

Model	Variable	Estimate (SE)	p-Value
PN	(Intercept)	8.682 (1.172)	<0.001
	Intensity (1)	2.489 (0.498)	<0.001
	Intensity (2)	2.654 (0.498)	<0.001
	Clone (747210)	2.445 (1.172)	0.082
	Clone (915319)	2.320 (1.172)	0.095
	Clone (DN2)	-1.077 (1.172)	0.394
	Week (3)	-0.090 (0.462)	0.846
	Week (4)	-0.123 (0.462)	0.790
	Week (5)	5.419 (0.462)	<0.001
TNC	(Intercept)	18.305 (1.449)	<0.001
	Root diameter	-0.135 (0.038)	0.001
	Intensity (1)	-3.105 (0.951)	0.002
	Intensity (2)	-4.173 (0.948)	<0.001
DELTA_1	(Intercept)	-26.233 (0.164)	<0.001
	Intensity (1)	-0.973 (0.162)	<0.001
	Intensity (2)	-1.219 (0.162)	<0.001
DELTA_2	(Intercept)	-28.842 (0.154)	<0.001
	Clone (747210)	1.390 (0.188)	<0.001
	Clone (915319)	1.568 (0.198)	<0.001
	Clone (DN2)	1.659 (0.204)	<0.001
	Intensity (2)	0.072 (0.092)	0.440
	Age (S ₂)	0.318 (0.103)	0.004
	Intensity2: age S ₂	-0.681 (0.133)	<0.001

Notes: SE is standard error of the mean, Intensity is the pruning intensity (1 = 1/3, 2 = 2/3 crown length), Clone is the type of clone, Season is the pruning season, Week is the week of measurement, Age is the age of leaf (S₂ = formed after pruning). Letters or numbers indicated in brackets indicate the type considered by the model. Combined with the values (column estimate), it allows to calculate the predicted values, for example in model PN, instantaneous net photosynthesis for Clone (747210) pruned at Intensity (1) and measured at week (2) was: $8.682 + 2.489 + 2.445$.

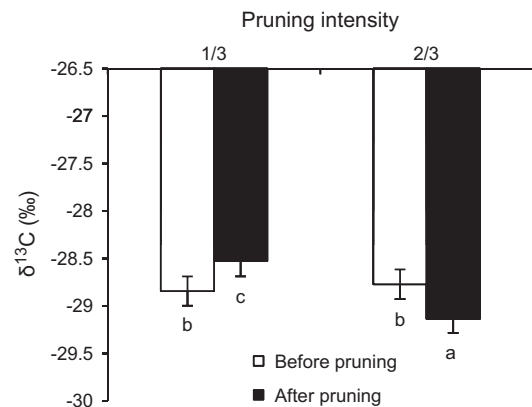


Fig. 4. Predictions for leaf carbon isotopic discrimination ($\delta^{13}\text{C}$) of summer-pruned trees at 1/3 and 2/3 pruning intensities. Errors bars are standard errors of the mean. Bars labeled with the same letter are not significantly different (Tukey's test at $p < 0.05$). Note that values were computed for clone 1079.

conductance of pruned trees was greater than that of unpruned trees, and the increase was greater for summer-pruned trees at intensity 2/3 (Fig. 5), giving a significant interaction between pruning season and intensity (Table 4).

4.6. Leaf nitrogen concentrations

Summer-pruned trees had greater leaf nitrogen concentrations compared to trees pruned in fall ($p = 0.022$) and spring ($p < 0.001$). Leaf nitrogen concentrations increased with pruning intensity for clones 1079 and DN2 while they were similar

Table 4

Best fit model chosen with backward selection for stomatal conductance (G_s) analysis of variance. Statistically significant values ($p < 0.05$) are given in bold.

Variable	G_s	
	Estimate (SE)	p-Value
(Intercept)	5.114 (0.148)	<0.001
Intensity (1)	0.250 (0.077)	0.002
Intensity (2)	0.326 (0.077)	<0.001
Week (3)	−0.153 (0.095)	0.007
Week (4)	−0.177 (0.056)	0.002
Week (5)	0.582 (0.056)	<0.001
Season (spring)	−0.080 (0.077)	0.302
Season (summer)	−0.024 (0.077)	0.755
Intensity (1):season (spring)	0.131 (0.110)	0.240
Intensity (2):season (spring)	0.105 (0.111)	0.347
Intensity (1):season (summer)	0.163 (0.108)	0.137
Intensity (2):season (summer)	0.360 (0.109)	0.001

Notes: SE is standard error of the mean, Intensity is the pruning intensity (1 = 1/3, 2 = 2/3 of crown length), Season is the pruning season, Intensity:season is the interaction between pruning intensity and pruning season. Letters or numbers indicated in brackets indicate the type considered by the model. Combined with the values (column estimate) it allows to calculate the predicted values, for example stomatal conductance for a tree pruned in season (spring) at intensity (1) and measured at week (3) was: $5.114 + 2489 + 0.250 - 0.153 - 0.080 + 0.131$.

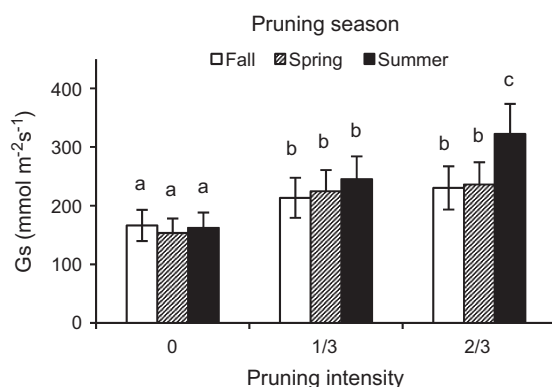


Fig. 5. Predictions for stomatal conductance (G_s) across pruning intensities and seasons. Errors bars are standard errors of the mean. Bars labeled with the same letter are not significantly different (Tukey's test at $p < 0.05$). Note that values were computed for week 2 (second week of gas exchange measurements).

between all pruning intensities for clones 747210 and 915319, giving a significant interaction between clone and intensity (Table 5, Fig. 6).

5. Discussion

Pruning at intensity 2/3 reduced height and dbh by 4.9% and 4.8%, respectively, while only height growth of 1/3 pruned trees (−3.2%) was reduced after two growing seasons (Fig. 2). Decreases in stem growth of severely pruned trees have often been reported (Funk, 1979; Krinard, 1979; Margolis et al., 1988; Uotila and Mustonen, 1994; Neilsen and Pinkard, 2003). The absence of a significant dbh decrease at intensity 1/3 is probably due to an increase in photosynthesis in the remaining leaves of pruned trees (Fig. 3a), illustrating the phenomenon known as compensatory photosynthesis (Nowak and Caldwell, 1984). An increase in net photosynthesis is commonly observed after pruning or defoliation (Heichel and Turner, 1983; Nowak and Caldwell, 1984; Pinkard and Beadle, 1998b; Medhurst et al., 2006). This increase in net photosynthesis was similar for trees pruned at 1/3 and 2/3 (+22.3% and +23.4%, respectively; Fig. 3a). Although compensatory photosynthesis should be proportional to the amount of foliage removed

Table 5

Best fit model chosen with backward selection for leaf nitrogen concentration (N) analysis of variance. Statistically significant values ($p < 0.05$) are given in bold.

Variable	N	
	Estimate (SE)	p-Value
(Intercept)	1.787 (0.047)	<0.001
Clone (747210)	0.369 (0.063)	0.001
Clone (915319)	0.244 (0.063)	0.008
Clone (DN2)	0.255 (0.063)	0.007
Intensity (1)	0.164 (0.059)	0.006
Intensity (2)	0.317 (0.059)	<0.001
Season (spring)	−0.032 (0.029)	0.267
Season (summer)	0.076 (0.029)	0.010
Clone (747210):intensity (1)	−0.106 (0.082)	0.199
Clone (915319):intensity (1)	−0.073 (0.082)	0.374
Clone (DN2):intensity (1)	0.057 (0.082)	0.490
Clone (747210):intensity (2)	−0.218 (0.083)	0.010
Clone (915319):intensity (2)	−0.152 (0.082)	0.067
Clone (DN2):intensity (2)	0.079 (0.082)	0.338

Notes: SE is standard error of the mean, Clone is the type of clone, Intensity is the pruning intensity (1 = 1/3 while 2 = 2/3), Season is the pruning season, Clone: Intensity is the interaction between the type of clone and pruning intensity. Letters or numbers indicated in brackets indicate the type considered by the model. Combined with the values (column estimate) it allows to calculate the predicted values, for example leaf N concentration for Clone (747210) pruned at Intensity (1) in spring was: $1.787 + 0.369 + 0.164 + -0.032 - 0.106$.

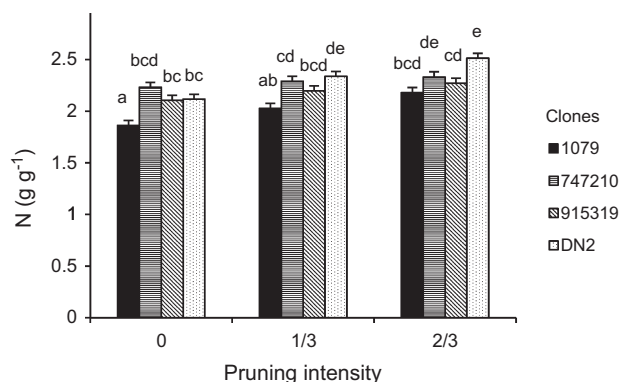


Fig. 6. Predictions for foliar nitrogen concentration (N) across pruning intensities for each clone. Bars labeled with the same letter are not significantly different (Tukey's test at $p < 0.05$). Note that values were computed for trees pruned in summer.

(Pinkard et al., 1998; Medhurst et al., 2006), there is a limit to the increase in stomatal conductance allowing for increased photosynthetic capacity. Indeed, Hart et al. (2000) showed that stomatal conductance of residual leaves increased with defoliation up to a certain point, after which it was similar in 50% and 98% defoliated aspen trees (*Populus tremuloides*). In our study, even if the increase in net photosynthesis was similar at both pruning intensities, the amount of residual foliage at intensity 2/3 was half the foliage of intensity 1/3, resulting in an overall lower photosynthetic capacity and reduced growth.

The increase in net photosynthesis of pruned trees could also be driven by the slight increases in leaf N concentrations of the remaining foliage (Fig. 6) (Trumble et al., 1993). The same amount of N is accumulated in a lesser number of leaves, resulting in higher concentrations in the remaining foliage, allowing increased CO_2 fixation rates since N is an essential component of chlorophyll, proteins and amino acids required for photosynthesis (Kozłowski and Pallardy, 1997). However, numerous studies have concluded that the increase in leaf N concentrations was not responsible for increased net photosynthesis of pruned trees (Reich et al., 1993;

Pinkard et al., 1998; Medhurst et al., 2006) but rather that it directly resulted from modified root-shoot ratios (McNaughton, 1983). Turnbull et al. (2007) observed that the N loaded into leaves in response to pruning was not reconverted in either Rubisco or chlorophyll and could be retained in a form requiring little maintenance, suggesting that the increase in photosynthetic rates created by pruning was not a result of an increase in foliar N concentrations, but rather due to the observed increased stomatal conductance. This is in accordance with our results that showed an increase in stomatal conductance of residual leaves of pruned trees (Fig. 5). Indeed, photosynthesis rates are largely controlled by stomata limitations (Farquhar and Sharkey, 1982). Moreover, greater stomatal conductance indicate that stomata remained more open, allowing trees to discriminate more against $^{13}\text{CO}_2$, which is in agreement with the decrease in $\delta^{13}\text{C}$ values that we found in pruned trees (Fig. 3c). A similar observation was reported by Larchevêque et al. (2011) who observed that *Populus balsamifera* trees with more negative $\delta^{13}\text{C}$ values maintained higher stomatal conductance.

Carbon isotopic composition of leaves can be used as a drought stress indicator since trees under moderate water deficit will close their stomata, resulting in intercellular spaces enriched in $^{13}\text{CO}_2$ (Farquhar et al., 1989). Plants growing under chronic drought conditions thus tend to have higher $\delta^{13}\text{C}$ values than those that are well watered (Kozłowski and Pallardy, 1997). Our $\delta^{13}\text{C}$ measurements suggest that leaves from unpruned trees were more water-stressed than those of pruned trees, which is reflected by less negative $\delta^{13}\text{C}$ values (Fig. 3c). We thus suggest that pruning improved trees' water status, likely because of a reduced transpiration surface.

Pruning reduced root TNC concentrations, either by reducing the photosynthetic apparatus and/or by triggering new foliage growth (Tschaplinski and Blake, 1995). We collected root samples in late summer, when TNC concentrations should have reached their maximum levels (DesRochers et al., 2002; Landhausser and Lieffers, 2003). Root TNC are allocated in priority to maintenance respiration, then to growth respiration in order to provide the energy needed to keep existing tissues healthy and synthesize new tissues (Kozłowski and Pallardy, 1997). This means that the compensatory photosynthesis we observed was not enough to restore root TNC reserves of pruned trees, especially at intensity 2/3. Those trees will have fewer resources for leaf flushing on the next growing season, which may explain why growth of 2/3 pruned trees was even more reduced after the second growing season (Fig. 2).

The effect of pruning season was more moderate than that of pruning intensity effect. Pruning season has no effect on instantaneous net photosynthesis rates, roots TNC reserves, and $\delta^{13}\text{C}$ values, while summer pruning increased foliar nitrogen (Table 5). Greater foliar nitrogen concentrations for summer-pruned trees were unexpected since summer pruning removes a lot of green foliage compared to fall and spring pruning, which were done on leafless trees. We suppose that trees were able to reallocate more nitrogen from other tree parts to the remaining leaves (Rennenberg et al., 2010), probably because trees were physiologically active during the summer pruning, which may induce a stronger and more rapid response.

The leaf carbon isotopic ratios of newly-formed leaves from summer pruned trees (S_2) decreased as pruning intensity increased, also indicating a reduction in water stress with pruning. Interestingly, leaves formed before the summer pruning (S_1) had similar $\delta^{13}\text{C}$ values for both pruning intensities (Fig. 4), but still lower than that of unpruned trees (Fig. 3c). This indicates that old leaves (S_1) were still fixing carbon in their tissues after leaf expansion was completed, probably to maintain metabolic functions. Thus a good example of how $\delta^{13}\text{C}$ can be used as a water-use efficiency indicator integrated over the whole growing season

(DesRochers et al., 2007). In situations of water deficit, the reduction of foliage in pruned trees could avoid or delay stomata closure compared to unpruned trees by reducing the overall tree transpiration.

We measured time-related changes in photosynthetic rates up to 5 weeks after the summer pruning treatment, and observed a large increase at week five ($+5.42 \mu\text{mol m}^{-2} \text{s}^{-1}$). In parallel we observed a similar increase in stomatal conductance at week five ($+131.41 \text{ mmol m}^{-2} \text{s}^{-1}$). Since this increase was also observed in unpruned trees, we associated it to climatic factors rather than to a time-since-treatment effect. The magnitude and duration of the photosynthetic response to pruning is species specific (Pinkard and Beadle, 2000). Compensatory photosynthesis was observed for 5 weeks after defoliation in poplars (Bassman and Dickmann, 1982), approximately 3 months in *Acer rubrum* and *Quercus rubra* (Heichel and Turner, 1983) and in some cases, 16 months for *Eucalyptus nitens* (Pinkard et al., 1998).

Tree physiological processes are strongly regulated by genetic makeup (Dickmann et al., 2001), it is why poplar clones often differ in their physiological responses to various silvicultural treatments (Bassman and Zwier, 1991; Marron et al., 2002). Although there were some clonal variations in our study such as larger net photosynthesis for clones 915319 and 747210, the four clones responded similarly to pruning intensity and season.

6. Management implications

For planning purposes, foresters need to know the expected growth reductions, if any, following pruning. We found that compensatory photosynthesis allowed lightly pruned trees to maintain growth rates similar to unpruned trees. Pruned trees seemingly also had better drought resistance, which could be used as a tool against periodic drought. However, pruning decreased root TNC concentrations, which could turn into less vigorous trees under stressful conditions. Regarding the season during which pruning is done, we observed that summer pruned trees had increased leaf nitrogen, stomatal conductance and decreased leaf carbon isotopic ratios. Such conditions could allow trees to recover from pruning more rapidly than trees pruned in fall or spring. Hence our recommendation to produce clear wood maintaining high growth rates in young hybrid poplar plantations would be to prune 1/3 of crown length during summer.

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